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(54) **Process for preparing high-concentration mixtures of polyunsaturated fatty acids and of their esters from oils of animal and/or vegetable origin, so obtained mixtures, and their use for prophylactic or therapeutical purposes**

(57) A process essentially based on the use of molecular distillation, for the preparation of polyunsaturated fatty acids, e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and of their ethyl esters, from oils of animal and vegetable origin is disclosed, which is particularly suitable for large-scale industrial productions. By this process, complexes constituted by EPA and DHA, or by their ethyl esters, with a total concentration not lower than 90% can be obtained, and these mixtures are used for various purposes, ranging from dietetic-alimentary uses to typically pharmaceutical uses for the management of a very large number of alterations, malfunctions, diseases and pathologies.

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TITLE:

PROCESS FOR PREPARING HIGH-CONCENTRATION MIXTURES OF POLY-
UNSATURATED FATTY ACIDS AND OF THEIR ESTERS FROM OILS OF
ANIMAL AND/OR VEGETABLE ORIGIN, SO OBTAINED MIXTURES, AND
THEIR USE FOR PROPHYLACTIC OR THERAPEUTICAL PURPOSES

5

The present invention relates to a process for
preparing a high-concentration mixture of eicosapentaenoic
acid and docosahexaenoic acid, and of their esters, by
starting from oils of various animal and/or vegetable
10 origins, as well as to the so obtained mixtures, and to
their use for prophylactic or therapeutical purposes.

The process of the present invention is furthermore
suitable for deodourising and deacidifying the same oils, in
view of a possible dietetical or alimentary use thereof.

15

It is known by now that the polyunsaturated fatty acids

play an important role in human being physiology, because they perform, in particular, two roles; a structural role, as constituents of the phospholipids of the cellular membranes, and a functional role, as precursors of
5 prostaglandins.

The fatty acids belonging to the family of α -linolenic acid perform in fact a basic task for the development and function of brain, retina and gonads, as well as for the formation of PGI_3 and TxA_3 , extremely important factors for
10 platelet agglutination preventive effect.

Among these, in particular, important are the long-chain members of ω -3 family, i.e., eicosapentaenoic acid ($20:5\omega$ -3), or EPA, and docosahexaenoic acid ($22:6\omega$ -3), or DHA, which derive from the desaturation and chain extension
15 of α -linolenic acid, thanks to the intervention of the relevant enzymes (Δ -desaturases).

EPA, as a precursor of both PGI_3 and TxA_3 , performs an platelet agglutination prevention action and an antithrombotic effect which can be reconducted to an
20 inhibition of cyclooxygenase (an aspirin-like effect) and/or to the competition with arachidonic acid for this enzyme, with a consequent decreased synthesis of PGE_2 and TxA_2 , well-known platelet agglutinants.

DHA is the most important component of brain lipids in
25 man and is present at high concentrations in the

phospholipids of the synaptic membranes, a fact, this, which makes researchers suppose DHA to play a role in the transmission of nervous impulse.

Furthermore, inasmuch as it is a structural element of
5 platelet cell, DHA indirectly plays, by increasing platelet fluidity, an important role in antithrombotic action.

Recent studies on man evidenced a decrease of Δ -6-desaturase enzyme with increasing age (after 35 years of age); as a consequence, an endogenous deficiency could occur
10 of above said acids, which therefore should be administered by means of the diet, or by means of suitable compositions. However, to date several practical difficulties have prevented a wide use of said acids to be made in therapy or as alimentary integrators, a use which, on the other hand,
15 would be highly desirable in view of the above reported biochemical and pharmacological background. Such difficulties are mainly related to the extraction of said acids from fish oils, their purification and concentration up to suitable values for a pharmaceutical use, and their
20 odourisation.

Although many methods have been proposed and published in the past, the above cited objectives have not been reached to a satisfactory extent yet, as, among others, the still now limited use of EPA and/or DHA demonstrates,
25 notwithstanding their considerable potentialities as drugs

or alimentary integrators. The methods known to date, based on different techniques, such as degreasing, counter-current extraction, urea addition, liquid chromatography, distillation, lead to rather low yields and to easily perishable products if exposed to light or to atmosphere. Furthermore, most known methods aim at purifying eicosapentaenoic acid only, to the detriment of other useful unsaturated fatty acids, such as DHA.

For example, U.S. patent 4,377,526 discloses a process for purifying EPA, or its esters, which comprises a treatment with urea, followed by fractional distillation. By such a method, percentages of EPA higher than 70% are obtained, whilst DHA remains present as a residue (3-5%) only.

Furthermore, as far as the present Applicant knows to date, all patented processes relating to the production of the same, or of similar, products, use a more or less complex combination of chemical or physical operations, such as, e.g., the use of urea for the preferential precipitation of less unsaturated acids (WO 87/03899, JP 57-187397), or extractions with supercritical fluids (JP 60-214757, IP 60-115698).

In order to reach high titers of DHA acids, or of esters thereof, in other patents also chromatography is used, with various chromatographic beds which range from

silica gel up to low-polarity copolymers (JP 61-291540, JP 61-037752, JP 58-109444, GB 2090529).

On the other hand, in those patents in which the molecular distillation is used (as, e.g., JP-113099), this is not the characterizing step of the process, but is simply used as a means for a rough purification during the processing.

The process of the present invention is exclusively based, apart from the common hydrolysis of triglycerides in order to obtain the acids, on the technique of molecular distillation. The molecular distillation is used by suitably changing the operating conditions, in order to obtain the whole range of products of the present invention, without any other chemical or physical treatments.

The object of the present invention is hence a method for extracting DHA and EPA ethyl esters and free fatty acids from raw oils of various kinds with high yields, under conditions easily applicable in the industrial field, and leading to a stable and odourless product, which can be used in human therapy both as a pharmaceutical and as a dietetic and alimentary product.

Furthermore, such a process only requires a vary small number of chemical treatments, in that it is substantially based on the articulated use of a technology, i.e., molecular distillation, which, owing to its operating

conditions, secures the highest protection of the products of the invention. In fact, these products are known to be very subject to undergo phenomena of chemical and thermal degradation. Finally, from the standpoint of the industrial
5 economic feasibility, molecular distillation is per se suitable for a continuous production, with extremely low operating costs.

The only use of molecular distillation in the process of the present invention makes it possible the following to
10 be obtained:

- 1) high quality products, also because they are not submitted to a too large number of chemical processes;
- 2) products at even very high concentrations, which may reach, in case of EPA DHA mixture, a value of 90%, and,
15 in case of DHA alone, 96%; these concentrations are considerably higher than as claimed in corresponding prior patents;
- 3) products at different titers for different uses, ranging from dietetic-alimentary uses to the typically
20 pharmaceutical use, by simply suitably varying the parameters of molecular distillation only;
- 4) the process according to the present invention is particularly suitable for an industrial production, contrarily to many of above cited patents, whose
25 implementation from a laboratory level to the industrial

level is much more expensive and problematic;

5) a further advantage is that, in case the ethyl esters have to be obtained, such a preparation can be carried out as the last processing step, by converting the acid products, at a suitable concentration and titer, into the corresponding esters. This is a considerable advantage from the view point of the reduction of the industrial production costs, and furthermore provides a process which is not disclosed in any of prior patents;

6) the process of the present invention is furthermore ideal for an industrial production, because, if the necessary equipment is available, it can be carried out in continuous mode, with a minimum use of labour and at the highest production level.

The high titers (up to 90%) and the considerable degree of purity of the EPA/DHA mixtures obtained by means of the process according to the present invention make it possible the pharmaceutical effects to be better pointed out, which derive from the administration of poly-unsaturated acids of U3 series, and, in particular, of EPA/DHA.

Having high concentrations of EPA/DHA, on one hand lower-weight, smaller-size pharmaceutical forms can be prepared, which are easier to ingest or administer, and, on the other hand, the number of daily intakes or administrations can be reduced.

The typical characteristics of EPA/DHA products of the present invention make it hence possible a greater therapeutical and formulation advantage to be attained in hyperlipemiae and therewith correlated pathologies, in thromboses, in platelet agglutination, in cardiac infarction, in hypertension, as anticoagulants, in prevention of atherosclerosis, in cerebral infarction, in lesions and occlusions caused by vasomotor spasms, in diabetes and its complications, in acute and chronic inflammations, in self-immune syndromes, in preventing the side effects at gastroenteric level of non-steroid anti-inflammatory agents, in tumor prevention.

The ratio of EPA concentration to DHA concentration changes according to the natural contents of the organism from which both compounds are extracted (e.g., various fish species, fish oils, crustaceans, sea weeds, and so forth).

The therapeutical properties of mixtures prevailingly containing EPA/DHA, or of mixtures containing, besides other poly-unsaturated fatty acids, also EPA/DHA, have been described in the past in several patents, and in particular: in the treatment of thromboses, in hypercholesterolemiae, in myocardial ischemia (WO 87/03899), in the prevention of arteriosclerosis, in cerebral infarction, in hyperlipemiae, in cardiac infarction (EP-A1-0 228 314), in the prophylaxis of atherosclerosis, as antithrombotic, as antihypertensive

(JP 62-091188), in thrombotic pathologies, in platelet agglutination, in self-immune syndromes, in acute and chronic inflammations, in atherosclerosis, cardiac infarction, in venous thromboses, in hyperlipemic states, in
5 hypertension, in lesions and occlusions originated by vasomotor spasms, in diabetes (WO 87/02247), in the prevention of the side effects of non-steroid anti-inflammatory agents (EP-A1-0 195 570), in the prophylaxis and management of diabetes complications (JP 60-248610), in
10 hypercholesterolemiae, in hypertriglyceridemiae (DE 34 38 630); as anticoagulants, in hypercholesterolemiae (BE 899 184). Furthermore, both EPA and DHA have an influence on the metabolism of poly-unsaturated fatty acids, promoting the formation of products endowed with a high biological
15 activity, i.e., the ecosanoids, which are active in tumor prevention.

Such activities were evidenced by prevailingly using poly-unsaturated fatty acids of ω 3 series, precursors of EPA
20 and DHA (JP 57-187397 and BE 897 806).

20 The preparations, whose references have been hereinabove cited, are often true mixtures of poly-unsaturated fatty acids prevailingly belonging to ω 3 series, and however, the EPA/DHA concentrations used are always considerably lower than those reached by means of the
25 process according to the present invention.

DHA, a highly unsaturated, long-chain fatty acid, belongs to the series denominated as "W3". Differently from what occurs in lower animal species, wherein both eicosahexaenoic acid (EPA) and DHA are present, in man only
5 traces of EPA, and high concentrations of DHA are found.

DHA is present in exclusively esterified form in membrane glycerophospholipids, and, in particular, in some districts, such as the CNS, in synaptic membranes and in retinal cells.

10 To the poly-unsaturated fatty acids belonging to W3 series, and to EPA, metabolic precursors of DHA, an extremely high number of biological and therapeutical activities have been attributed.

In the metabolic pathway starting from α -linolenic acid
15 and leading to DHA, the administration of EPA does not lead, except for small amounts, to the conversion into DHA, whilst a portion of administered DHA is converted back into EPA.

In fact, the ingestion of DHA, in ester form, and/or as the free acid, significantly increases both DHA and EPA
20 levels in plasmatic phospholipids (Hiroi et al., 1978).

Thus, DHA, besides performing its own task, would also ensure, by being converted back into EPA, the biological actions typical of EPA.

In prior patents, several therapeutical activities have
25 been claimed for mixtures of poly-unsaturated fatty acids of

W3 series, to which DHA belongs, and, in particular, therapeutic activities have been claimed in hyperlipemiae and therewith correlated pathologies, in thromboses, in platelet agglutination, in cardiac infarction, in hypertension, as anticoagulants, in atherosclerosis prevention, in cerebral infarction, in lesions and occlusions caused by vasomotor spasms, in diabetes and its complications, in acute and chronic inflammations, in self-immune syndromes, in preventing the side effects at the gastroenteric level of non-steroid anti-inflammatory agents, and in tumor prevention (WO 87/03899, EP-A1-0 228 314, JP 62-091188, WO 87/02247, EP-A1-0 195 570, JP 60-248610, DE 34 38 630, BE 899 184, JP 57-187397, BE 897 806).

DHA, as a single substance, was evaluated in therapy as a platelet agglutination preventive agent, and an use thereof in the prophylaxis of thrombotic processes was proposed (GB 2,098,065, GB-2,090,529).

In reality, the DHA used in the prior studies does not seem to have been as highly concentrated as DHA obtained by means of the process according to the present invention.

Furthermore, the process of extraction of the present invention, by means of molecular distillation, without either chemical or physical treatments, characterizes the obtained DHA with a high purity degree, as compared to the previously obtained products.

By means of different experimental models, the activity of highly concentrated (96%) and purified DHA in hyperlipemiae was pointed out. In fact, the administration of DHA reduced, to a meaningful extent, the experimentally
5 induced high levels of cholesterol and triglycerides.

On considering the obtained results, on the basis of the functions which DHA performs inside the organism, and as a consequence of the phenomena observed in various districts when DHA is administered, its characteristics and
10 therapeutical peculiarities can be summarized as follows: in the treatment and prophylaxis of dislipemic diseases and therewith connected pathologies, such as hyperlipoproteinemiae, hypercholesterolemiae, hypertriglyceridemiae, in the alterations of fat metabolism,
15 in damages to vessels caused by cholesterol, in atherosclerosis, in xanthomas, in diabetic retinopathy, in the prevention of thrombus formation, in prevention of aortal and coronary arteriosclerosis, as a coadjuvant in those diseases which may originate manifestations of
20 hyperlipoproteinemiae (diabetes mellitus, hypothyroidism, uraemia, and so forth), in cardiac infarction, in platelet agglutination, in hypertension, in anticoagulant therapy, in cerebral infarction, in acute and chronic inflammations, in diabetes, in self-immune syndromes, in the prevention of the
25 side effects caused by non-steroid anti-inflammatory agents,

in tumor prevention, in retinopathies with visual deficit,
in ceroidoses, in the processes relevant to learning and
ageing.

The process according to the present invention is
5 disclosed now in detail, and one will thus see that by means
of said process, those purposes and advantages which have
been hereinabove outlined can be fully achieved.

An alkaline hydrolysis (NaOH) of the raw oil is
performed up to the complete breakdown of the triglycerides.

10 The solid soap formed is collected and is immediately
acidified with mineral acid in an aqueous solution.

The formed acids are extracted with petroleum ether, up
to exhaustion. The extracts are combined with one another,
are thoroughly washed with water, and are concentrated up to
15 total solvent removal.

The resulting product is processed by exclusively using
molecular distillation, in such a way as to obtain the whole
range of products according to the present invention.

A) COMPLEX CONSTITUTED BY EPA AND DHA

20 A - 1 Total concentration comprised within the range of from
35 to 40%

A - 2 Total concentration comprised within the range of from
40 to 50%

A - 3 Total concentration comprised within the range of from
25 50 to 60%

A - 4 Total concentration comprised within the range of from
60 to 70%

A - 5 Total concentration comprised within the range of from
70 to 80%

5 A - 6 Total concentration comprised within the range of from
80 to 90%

B) PRODUCT CONSTITUTED BY DHA

B - 1 Total concentration 90%

B - 2 Total concentration 96%

10 - Process for obtaining A-1 product

The mixture of fatty acids obtained from the first step of the process (saponification) is submitted to molecular distillation, operating under a pressure of 10^{-3} mmHg, with a temperature of the evaporator of 110-120°C, in order to
15 remove the process impurities and the natural impurities, which constitute the residue.

The distillate maintains the concentration of EPA and DHA which was originally present in the starting oil (15-20% of EPA and 15-20% of DHA, in case of a fish oil), and is
20 free from above-said impurities; it constitutes, per se, the A-1 end product, and is the starting material for subsequent A-2, A-3, A-4 products.

- Process for obtaining A-2 product

A-1 product is submitted to molecular distillation,
25 under a pressure of 10^{-3} mmHg and with an evaporator

temperature of 50°C. The residue is constituted by A-2 product, and the increase in EPA and DHA titer takes place to the detriment of the lower molecular-weight acids (C₁₆ and C₁₈), which constitute the distillate.

5 - Process for obtaining A-3 product

A-1 product is submitted to molecular distillation under the above-said conditions, except for evaporator temperature, which is increased to 60°C. The residue is constituted by A-3 product, and the increase in EPA and DHA
10 titer takes place to the detriment of the lower molecular-weight acids (C₁₆ and C₁₈), which constitute the distillate.

- Process for obtaining A-4 product

A-1 product is submitted to molecular distillation under the above-said conditions, except for evaporator
15 temperature, which is increased to 70°C. The residue is constituted by A-4 product, and the increase in EPA and DHA titer takes place to the detriment of the lower molecular-weight acids (C₁₆ and C₁₈), which constitute the distillate.

- Process for obtaining A-5 product

20 A-4 product is submitted to molecular distillation under the above-said conditions, except for evaporator temperature, which is increased to 75°C. The residue is constituted by A-5 product, and the increase in EPA and DHA
25 titer takes place to the detriment of the lower molecular-weight acids (C₁₆-C₁₈ and lower-unsaturated-C₂₀), which

constitute the distillate.

- Process for obtaining A-6 product

A-5 product is submitted to molecular distillation under the above-said conditions, except for evaporator temperature, which is of 80°C. The residue is constituted by A-6 product, and the increase in EPA and DHA titer takes place to the detriment of the lower molecular-weight acids (C₁₆-C₁₈ and lower-unsaturated-C₂₀), which constitute the distillate.

10 - Process for obtaining B-1 product

A-6 product is submitted to a double molecular distillation under the above-said conditions, except for evaporator temperature, which is of 85°C. The residue is constituted by B-1 product (DHA 90%), and the distillate is mainly constituted by EPA and minor amounts of other acids.

- Process for obtaining B-2 product

B-1 product is submitted to molecular distillation under the same condition as used for B-1, with the evaporator temperature being of 85°C. The residue is constituted by 96% of DHA, and the distillate is mainly constituted by EPA and minor amounts of other acids.

The general process for producing the ethyl esters of the above products is disclosed now.

The esterification process is the same for all above disclosed products, and consists in treating the fatty acids

with ethanol in an acidic medium, by means of the usual techniques.

To the reaction product, an equal volume of water is added, and the whole mixture is extracted with petroleum
5 ether.

After being dried, the ethereal solution is evaporated up to total solvent disappearance.

The obtained product is submitted to molecular distillation, operating under the hereinabove disclosed
10 conditions, with the temperature of the evaporator being of 100°C, for the total elimination of any process impurities.

C) COMPLEX CONSTITUTED BY EPA AND DHA ETHYL ESTERS

C - 1 Total concentration comprised within the range of from
35 to 40%

15 C - 2 Total concentration comprised within the range of from
40 to 50%

C - 3 Total concentration comprised within the range of from
50 to 60%

20 C - 4 Total concentration comprised within the range of from
60 to 70%

C - 5 Total concentration comprised within the range of from
70 to 80%

C - 6 Total concentration comprised within the range of from
80 to 90%

25 D) PRODUCT CONSTITUTED BY DHA ETHYL ESTER

D - 1 Total concentration 90%

D - 2 Total concentration 96%

- Process for obtaining the ethyl esters of C-1 product

5 A-1 product is submitted to the hereinabove disclosed general esterification process.

- Process for obtaining the ethyl esters of C-2 product

A-2 product is submitted to the hereinabove disclosed general esterification process.

- Process for obtaining the ethyl esters of C-3 product

10 A-3 product is submitted to the hereinabove disclosed general esterification process.

- Process for obtaining the ethyl esters of C-4 product

A-4 product is submitted to the hereinabove disclosed general esterification process.

15 - Process for obtaining the ethyl esters of C-5 product

A-5 product is submitted to the hereinabove disclosed general esterification process.

- Process for obtaining the ethyl esters of C-6 product

20 A-6 product is submitted to the hereinabove disclosed general esterification process.

- Process for obtaining the ethyl esters of D-1 product

B-1 product is submitted to the hereinabove disclosed general esterification process.

- Process for obtaining the ethyl esters of D-2 product

25 B-2 product is submitted to the hereinabove disclosed

general esterification process.

The following examples are given in order to illustrate the process of the present invention in greater detail, and in no way they should be construed as being limitative of the scope of protection of the present invention.

Example No. 1

100 kg of raw oil is treated at boiling temperature, under nitrogen, with 150 litres of water and NaOH (approximately 3 mol per triglyceride mol).

10 The obtained soap is collected and acidified, until the aqueous solution turns to an acidic pH value. The formed acids are extracted with three portions of petroleum ether, for a total of 150 litres.

The extracts are washed with water until neutral, and 15 are concentrated up to total solvent removal.

The so-obtained product (A-1) is charged to the molecular still and is distilled, by operating under one of the above-said operating conditions.

In case, e.g., obtaining A-3 product is desired, A-1 20 will be submitted to molecular distillation with the evaporator temperature being of 60°C. A distillate to be discharged, and a residue containing 60-65% of EPA/DHA, and whose volume corresponds to 30% of injected volume, will be obtained.

Example No. 2

In case, e.g., A-6 product is desired, A-5 product will be submitted to molecular distillation, by operating under the hereinabove cited conditions, with the evaporator temperature being of 80°C. A residue containing 83% of EPA/DHA, and whose volume corresponds to 18% of the injected volume, will be obtained.

Example No. 3

The process for obtaining the product constituted by DHA is carried out by operating in the same way as disclosed in previous examples.

By treating A-6 product by a double molecular distillation, with the temperature of the evaporator being of 85°C, a residue containing 90% of DHA (B-1 product), whose volume is 54% of the injected volume, will be obtained.

Finally, the present Applicant points out that many modifications, variants, additions and/or replacements of elements, process steps and operating details can be supplied to the process of the present invention, without thereby departing from the spirit or from the purview of said invention, and without departing from the protecting scope thereof, as it is also defined in the hereto appended claims.

C l a i m s

1. Process for preparing high-concentrated mixtures of poly-unsaturated fatty acids and their esters, from oils of animal and/or vegetable origin, characterized in that the
5 raw oil is submitted to an alkaline hydrolysis, the solid soap so formed is acidified with a mineral acid in aqueous solution, the resulting mixture is extracted with petroleum ether up to exhaustion and then, after washing and concentration, the combined extracts are submitted to one or
10 more molecular distillation step(s), with the pressure and temperature parameters being suitably changed, in order to obtain the whole range of the desired end products.

2. Process according to claim 1, characterized in that the first molecular distillation is carried out under a
15 pressure of 10^{-3} mmHg, and at a temperature of the evaporator of approximately 110-120°C.

3. Process according to claim 1, characterized in that the subsequent molecular distillation steps are carried out at an increasing temperature of from 50 to approximately
20 80°C, in order to increase the titer of the poly-unsaturated fatty acids obtained, to the detriment of acids having progressively increasing molecular weight.

4. Process according to claim 1, characterized in that the poly-unsaturated fatty acids obtained are mainly
25 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

5. Process according to claim 4, characterized in that in order to obtain a residue nearly exclusively constituted by DHA at a high concentration, higher than 90%, the products from the previous steps of molecular distillation are submitted to a further molecular distillation at a higher temperature, not lower than approximately 85°C.

6. Process according to one or more of the preceding claims, characterized in that in order to prepare the ethyl esters of the poly-unsaturated fatty acids obtained from the molecular distillation, the fatty acids are treated with ethanol in an acidic medium, to the reaction product an equal volume of water is added, and the whole mixture is extracted with petroleum ether, the ethereal solution, after being dried, is evaporated up to total removal of the solvent, and the product is then submitted again to molecular distillation at a high temperature of approximately 100°C, in order that the process impurities are totall removed.

7. Mixture of poly-unsaturated fatty acids, whenever obtained by means of the process according to one or more of claim(s) 1-5.

8. Mixture of ethyl esters of poly-unsaturated fatty acids, whenever obtained by means of the process according to claim 6.

9. Mixture according to claim 7, characterized in that

the poly-unsaturated fatty acids are mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

10. Mixture according to claim 9, characterized in that the total concentration of the mixture is comprised within the range of from approximately 35 to approximately 90%, according to the number of steps of molecular distillation carried out.

11. Product essentially constituted by DHA at a total concentration of approximately 90%, whenever obtained by means of the process according to claim 5.

12. Mixture of ethyl esters of EPA and DHA, whenever obtained by means of the process according to claims 1-7.

13. Product constituted by DHA ethyl ester at a high concentration, of at least 90%, whenever obtained by means of the process according to claims 1-7.

14. Use of the products or mixtures according to claims 7-13 as dietetic-alimentary additives.

15. Use of the products or mixtures according to claims 7-3 as pharmacologically active substances, for prophylactic or therapeutical purposes.

16. Use of mixtures according to claims 7-10 and 12 for the treatment and prevention of hyperlipemiae and therewith correlated pathologies, in thromboses, in platelet agglutination, in cardiac infarction, in hypertension, as anticoagulants, in atherosclerosis prevention, in cerebral

infarction, in lesions and occlusions caused by vasomotor spasms, in diabetes and its complications, in acute and chronic inflammations, in self-immune syndromes, in preventing the side effects at gastroenteric level of non-steroid anti-inflammatory agents and in tumor prevention.

17. Use of DHA or of its ethyl ester according to claims 11 and 13 for the treatment and prevention of dislipemic diseases and therewith connected pathologies, such as hyperlipoproteinemiae, hypercholesterolemiae, hypertriglyceridemiae, alterations of fat metabolism, damages to vessels caused by cholesterol, atherosclerosis, xanthomas, diabetic retinopathy, in the prevention of thrombus formation, in prevention of aortal and coronary arteriosclerosis, as a coadjuvant in those diseases which may originate manifestations of hyperlipoproteinemiae (diabetes mellitus, hypothyroidism, uraemia, and so forth), in cardiac infarction, in platelet agglutination, in hypertension, in the anticoagulant therapy, in cerebral infarction, in acute and chronic inflammations, in diabetes, in self-immune syndromes, in the prevention of the side effects caused by non-steroid anti-inflammatories, in tumor prevention, in retinopathies with visual deficit, in ceroidoses, in the processes relevant to learning and ageing.

18. Process as claimed in Claim 1, substantially as described in any of the examples described herein.

19. A mixture as claimed in Claim 7, substantially as described in any one of the examples disclosed herein.